



## Interactive effects of peripheral and central administration of LPS with inhibition of CRF receptors on food intake in neonatal chicks

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### ABSTRACT

Anorexia is a part of the acute phase response (APR). Lipopolysaccharide (LPS) is frequently used to mimic APR and induces anorexia. The mechanism underlying anorexia associated with APR in chicks is not well understood. In the present study, the possible involvement of corticotrophin-releasing factor (CRF) on anorexic effects of LPS in neonatal chicks was investigated. For this aim, different doses of LPS were administered via both intracerebroventricular (ICV) and intraperitoneal (IP) routes in order to assess its effects on chick's food intake. Subsequently, the effect of ICV injection of astressin, a CRF receptor antagonist, on anorexia induced by ICV and IP administration of LPS was investigated. Food intake was significantly decreased following either central or systemic administration of LPS. ICV co -injection of astressin and LPS significantly diminished anorexic effects of central LPS. However, anorexia induced by peripheral LPS was not attenuated by central injection of astressin. These data indicated that the brain CRF receptors are involved in central LPS-induced anorexia in chicks.

### Keywords

*Lipopolysaccharide, Acute illness anorexia, Corticotrophin-releasing factor (CRF) receptors, Neonatal chicks*

### Abbreviations

APR: Acute phase response  
CRF: Corticotrophin-releasing factor  
LPS: Lipopolysaccharide  
COX2: Cyclooxygenase 2  
PG: Prostaglandin  
EP4: Prostaglandin E2 receptor 4  
PVN: Paraventricular nucleus  
ICV: Intracerebroventricular  
IP: Intraperitoneal

## Introduction

Energy homeostasis mechanisms are complicated and also in part different in animal species, including birds (1, 2). Similar to mammals, under physiological conditions hypothalamic nuclei play a crucial role in chick energy homeostasis (3). Hypothalamic homeostatic functions including appetitive and feeding behavior are extensively affected by immune agents (4, 5). Indeed, infectious challenges initiate acute-phase response (APR), a systemic defense mechanism, which is commonly reflected by immunological, physiological, and behavioral disturbances (6, 7). The behavioral changes are known as “sickness behavior,” and are represented by depression, changes in motivational state, fever and the decrease in food intake namely illness anorexia (8). Lipopolysaccharide (LPS), an endotoxin matter of cell surface of gram-negative bacteria, has been widely used as an experimental inflammatory model for evaluation of possible underlying mechanism(s) of anorexia in different species (9). LPS motivate expression of pro-inflammatory cytokines and anorexia-related agents, including corticotrophin-releasing factor (CRF) (10, 11). CRF plays an important role in stress responses such as changes in the hypothalamic-pituitary-adrenal axis, autonomic nervous system, immune system and behavior (12, 13). These actions of CRF are mediated through two receptor subtypes; CRF receptor 1 (CRF1) and CRF receptor 2 (CRF2)(14).

Several evidences have revealed inhibitory effects of CRF on food intake (15, 16). It has been shown that intracerebroventricular (ICV) administration of CRF

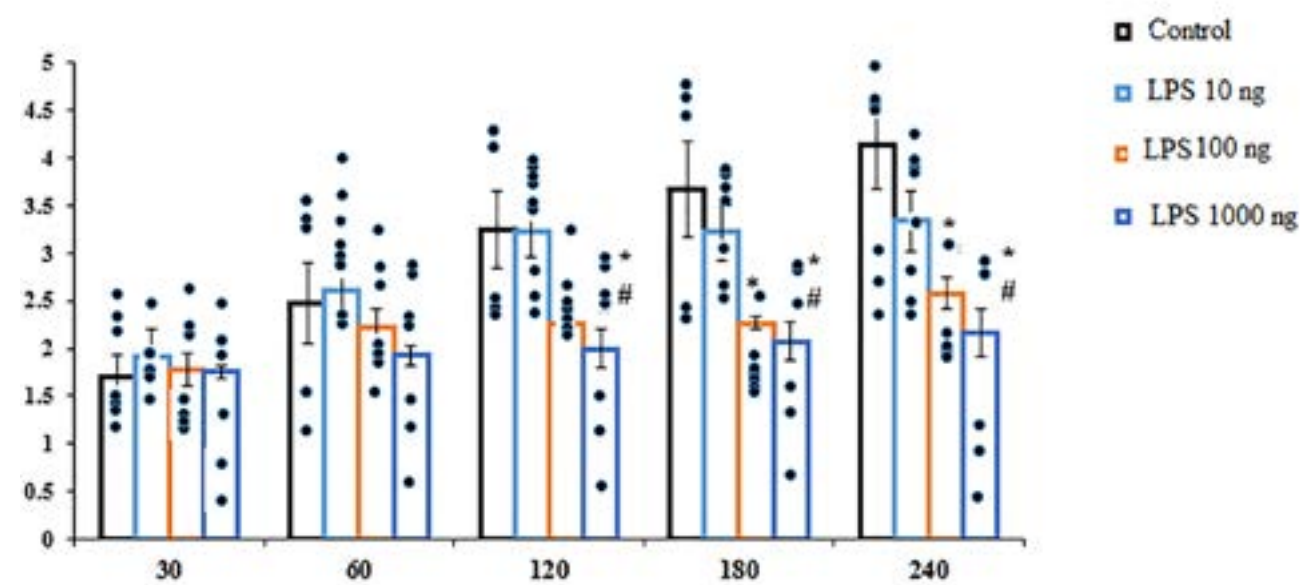
inhibits food intake in both mammals and chicks (17, 18). Saito et al (2005) indicated that the inhibitory effect of ghrelin on food intake was mediated by CRF in neonatal chicks (19).

Besides reported anorexic effects of CRF, none is known about the CRF involvement in anorexic effects of LPS in birds. Thus, the present study was conducted to evaluate the effect of ICV injection of Astressin as a nonselective CRF receptor antagonist on anorexic effects of LPS in chicks.

## Results

### Food intake response to central LPS

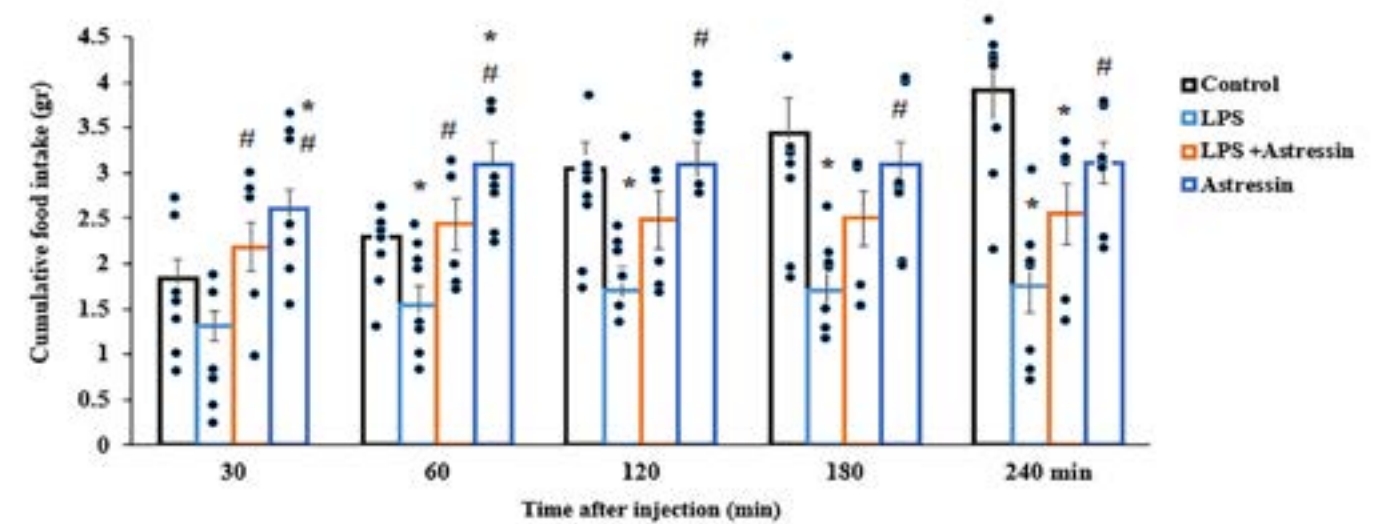
Time-course of chick's food intake injected ICV with different doses of LPS is presented in Fig 1. Cumulative food intake started to decrease the appetite of chicks treated with LPS (100 and 1000 ng) 30 min post injection and this suppression continued strongly, so that chicks almost did not consume food until the end of the experiment. However, this suppression was statistically significant from 120 min post injection and thereafter. The central effects of astressin (20 µg) and astressin co-injected with LPS (100 ng) on cumulative food intake of birds are represented in Figure 2. Food intake was not decreased by LPS plus astressin (except 240 min post injection), while it was significantly decreased by LPS alone compared to the control 60 to 240 min after injection. Also, astressin or LPS plus astressin, significantly increased food intake compared to the LPS group. All of these results show that anorexia induced by LPS is attenuated by the blockage of CRF receptors.



**Figure 1**  
Cumulative food intake following ICV injection of various doses of LPS in chicks. Values correspond to mean  $\pm$  S.E.M.

\*  $p < 0.05$  compared to control group

#  $p < 0.05$  compared to LPS 10 ng group

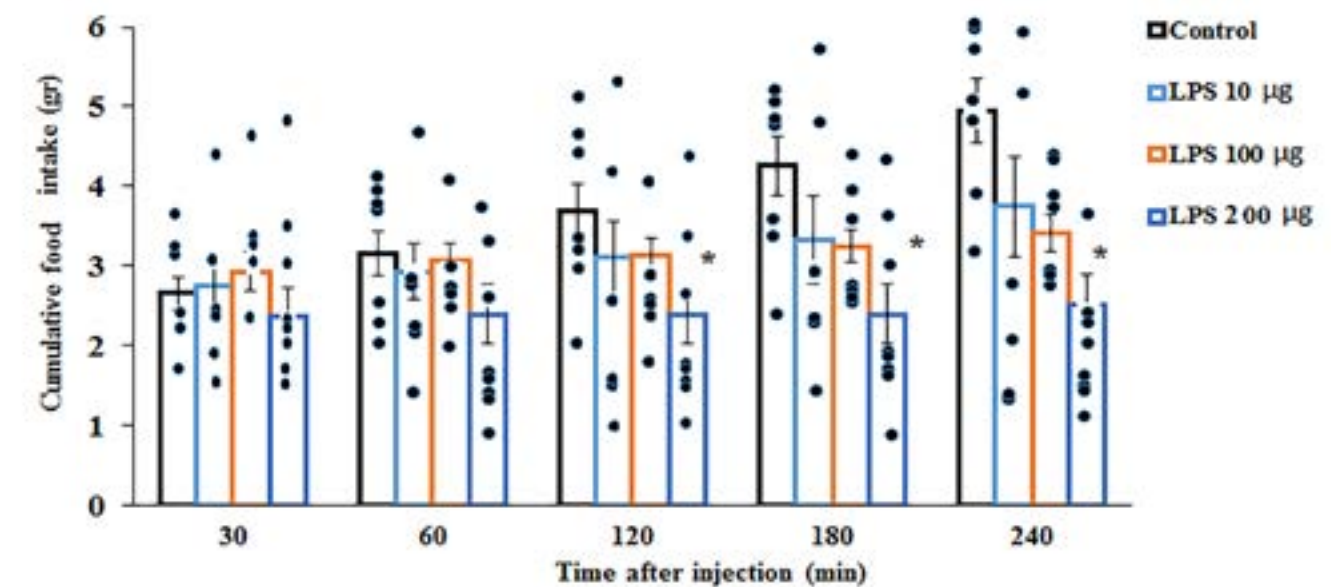


**Figure 2**

Cumulative food intake following ICV injection of LPS (100 ng) and LPS plus astressin (20 µg) in chicks. Values correspond to mean  $\pm$  S.E.M.

\*  $p < 0.05$  compared to control group

#  $p < 0.05$  compared to LPS group



**Figure 3**

Cumulative food intake following IP injection of LPS in chicks. Values correspond to mean  $\pm$  S.E.M.

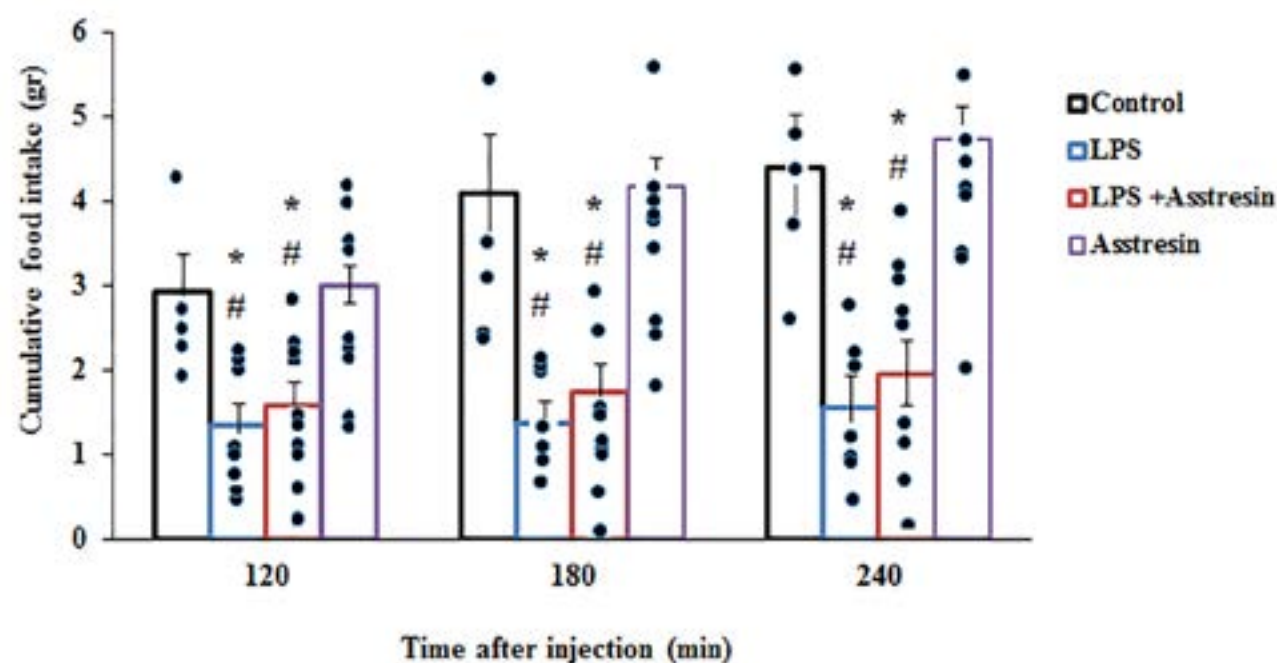
\*  $p < 0.05$  compared to control group

### Food intake response to peripheral LPS

Figure 3 shows the cumulative food intake of birds injected IP with different doses (10, 100 and 200 µg) of LPS. Food intake tended to decrease by all levels of LPS as a dose dependent manner. However, in chicks with 200 µg LPS, food intake was strongly sup-

pressed so that the birds did not eat nearly until the end of the experiment. However, this suppression was statistically significant from 120 min post injection and thereafter. The anorexia induced by IP injection of LPS was not attenuated by central astressin (both LPS and LPS plus astressin treated groups showed decreased food intake, while there was no significant





**Figure 4**  
Cumulative food intake following IP injection of either 0 or 200 µg LPS followed by ICV injection of 0 or 20 µg Astressin 90 min later in chicks. Values correspond to mean ± S.E.M.  
\*  $p < 0.05$  compared to control group  
#  $p < 0.05$  compared to Astressin group

difference between them)(Fig 4).

## Discussion

In this study we have shown that peripheral and central administration of LPS could strongly diminish the neonatal chicks' food consumption. The anorexic effects were initiated 30 min after LPS injection and enlarged with increasing doses (Figs 1 and 3). LPS from gram-negative bacterial cell walls are major promoters of the APR and reduced food intake in animals (20). In mammals, many of the physiological effects of LPS via acting on its recognition receptor toll like receptor 4 are mediated by pro-inflammatory cytokines, like interleukin (IL)-1, IL-6 and TNF- $\alpha$  which are released from activated cells of monocyte/macrophage lineage (21-24). Thus, Pro-inflammatory cytokines are major endogenous mediators of the acute illness anorexia. These cytokines activate cyclooxygenase 2 (COX2), an enzyme that facilitates the metabolism of arachidonic acid to prostaglandin (PG) E2 (25). In chickens, in agreement with our results, IP and ICV injections of LPS have been demonstrated to induce hyperthermia and anorexia and to increase corticosterone (26, 27). PGs have also been demonstrated to be involved in LPS-induced hyperthermia and anorexia in chickens (27). Chickens were

also injected with indomethacin (a COX2 inhibitor), peripherally or centrally following a challenge with IP injection of LPS. Pretreatment with indomethacin (injected IP but not ICV) significantly attenuated the LPS-induced anorexia (27). In addition, intravenous injection of LPS has been reported to increase plasma PGE2 concentrations in chickens (28). As it was mentioned before, we also investigated the effect of the blockade of CRF receptors by Astressin on anorexia induced by LPS. The results showed that centrally (not peripherally) LPS-induced anorexia is attenuated when the CRF receptors are blocked (Figs 2 and 4). Consistent with this result, it has been demonstrated in chicks that IL-1 and 3 activate stress axis, the key pathway for prostaglandin-induced fever, sickness behavior, and anorexia (29-31). In mammals, both CRF and cortisol influence the central mechanisms involved in the regulation of food intake (32). In chicks, several lines of evidence have shown that many anorectic agents exert their effects via CRF neurons. Indeed, the anorexigenic effects of ghrelin, glucagon like peptide -1,  $\alpha$ -melanocyte stimulating hormone, vasoactive intestinal peptide, pituitary adenylate cyclase-activated peptide, glucagon, and cholecystokinin (19, 33-37), are mediated by CRF. Although, mechanisms underlying LPS induced anorexia mediated by CRF is unknown in chicks, there are some indications

of this mechanism to be present in mammals. It has been reported that LPS induces the expression of CRF and prostaglandin E2 receptor 4 (EP4), and activates CRF neurons in the rat PVN (10, 38, 39). Pro-inflammatory cytokines may directly activate CRF neurons within PVN (40). Peripheral injections of LPS or IL-1  $\beta$  increase COX-2 and microsomal Prostaglandin E synthase-1 expression in blood brain barrier endothelial cells (41-43). PGE2 may directly act on its receptors, EP4 within PVN to release CRF (44). Evidences indicate that PGE2 released in response to LPS (and probably pro-inflammatory cytokines) may also act on serotonergic neurons to elicit anorexia. These evidences suggest that serotonergic neurons expressing EP3 receptors might be activated by PGE2 and project to areas of the hindbrain and forebrain that are involved in the control of food intake (45, 46). Pre-treatment with NS-398, a COX-2 inhibitor, reduced or eliminated LPS-induced c-Fos expression in several brain areas including the raphe complex, a source of serotonergic neurons (47, 48). Serotonergic neurons via their 2C receptors may act on PVN to release CRF (48). Recently, Zendejdel et al reported that pre-treatment with a 2C serotonin receptor antagonist significantly attenuated food intake suppression caused by LPS in chickens (49).

In this study, IP injection of LPS followed by ICV injection of Astressin couldn't attenuate LPS-induced anorexia effects. This discrepancy may be attributed to the difference in peripheral and central pathways of LPS action. In agreement to this, Johnson et al showed that central injection of LPS increases corticosterone plasma levels more than peripheral LPS, indicating that more CRF is released by central LPS (26).

In conclusion, current study revealed that both central and peripheral LPS strongly suppress food intake in chicks 30 min post injection and thereafter. The required amount of LPS for central suppression was about 1000 times lower than that required for peripheral suppression. Our results also identified that the CRF receptors are involved in the anorexic effect of central LPS in chicks. CRF has been shown to be a food intake inhibitor in chicks and many anorexigenic factors act through the CRF pathway in chicks. However, further studies are needed to clarify the CRF receptor subtypes involved in the above mentioned pathway in chicks.

## Material and methods

### Animals

One-day-old Ross broiler chicks were purchased from a local hatchery (Mahan Chicken Meat Production Complex, Kerman, Iran). All birds were given free access to a commercial feed and water and continuous lighting. The temperature and relative hu-

midity of the animal cage were maintained at  $30 \pm 1^\circ\text{C}$  and  $50 \pm 5\%$ , respectively. Animals were placed in individual cages, one day before the experiment. All efforts were made to decrease distress. The principles of working with animals were based on the recommendations of the ethics committee of Kerman University of Medical Sciences, Kerman.

### Drugs

LPS from *Salmonella typhimurium* (Sigma & Aldrich, USA) and Astressin (Tocris Bioscience, UK), a nonselective CRF antagonist, dissolved in sterile 0.85% NaCl plus 0.1% Evance Blue (Sigma & Aldrich, USA). Control animals received drug vehicle. All drugs were freshly prepared on each experimental day.

### Microinjections

ICV injection was performed according to Davis et al. method (50). Briefly, the head of the chick was inserted in a straining device which positioned a hole in a plate overlying the skull immediately over the right lateral ventricle. A microsyringe was then inserted into the right lateral ventricle through the hole and infusions were delivered in a total injection volume of 10 µl. This method requires no anesthesia and stress level of birds is insignificant (19, 51). At the end of each behavioral test, the animals were killed with intracardiac injection of sodium thiopental and their brain was removed. Validation of drug injection was verified by the presence of Evans blue in the right lateral ventricle. If an injection was not fixed in the correct location, the chicks' data were omitted from the analysis.

### Experimental procedure

This study was designed in four experiments. In experiment 1, chicks were given ICV injection of LPS at 0, 10, 100 and 1000 ng. Experiment 2 was conducted to determine the central effects of Astressin, as a CRF receptor antagonist, on LPS-induced change in chicks' food intake. Thus, the birds received ICV injection of LPS at 0 and 100 ng, Astressin at 20 µg and Astressin (20 µg) plus LPS (100 ng). In experiment 3, animals were given an intraperitoneal (IP) injection of LPS at 0, 10, 100 and 200 µg. Experiment 4 was similar to experiment 2 except that the chicks were given IP injection of either 0 or 200 µg LPS, then, 90 min later, they received ICV administration of either 0 or 20 µg Astressin. In all experiments, 6-day-old chicks were deprived of food for 3 h prior to injections in order to motivate and coordinate feeding. Cumulative food intake was measured at 30 to 240 min post injection. 9-12 chicks were used for each experimental group.

### Data analysis

Data was presented as means ± SEM. The results were evaluated statistically using ANOVA (IBM\*SPSS statistics\* version 23) followed by a post hoc Duncan's new multiple rang test (MRT). Differences were considered statistically significant when  $p < 0.05$ .

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## Author Contributions

Author contributions: Designed the experiments: H.J., M.A. Performed the experiments: M.S., A.S.H. Analyzed the data: M.Y., Research space and equip-

ment: M.A., Wrote the paper: R.K.

## Conflict of Interest

None.

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